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Studies on the Absorption, Residues and Metabolism of Cyromazine in Tomatoes

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Abstract: The residues and metabolism of radio-labeled cyromazine (*N*-cyclopropyl-1,3,5-triazine-2,4,6-triamine) formulated as a 750 g kg^{-1} WP ('Trigard' 75WP)® was studied in greenhouse tomatoes. Residues of cyromazine and its metabolites were remarkably persistent, with only a slight loss occurring within a 30-day period. Findings with tomatoes were similar to those with glass plates when exposed to cyromazine. Little volatilization occurred from either tomato or glass surfaces kept under greenhouse conditions. The only metabolite identified was melamine on both glass plates and tomatoes. Two other metabolites were found but were not identified.

Key words: cyromazine, residues, tomatoes

1 INTRODUCTION

Cyromazine (*N*-cyclopropyl-1,3,5-triazine-2,4,6-triamine) is an insect growth regulator registered in the United States for fly control ('Larvadex'®) in the poultry industry. It has also been utilized for prevention of 'fly strike' in sheep against *Lucilia cuprina* (Wied) in Australia and South Africa.[1] It has excellent growth-inhibitory properties against various flies[2–8] as well as fleas,[9] thrips[10] and the tobacco hornworm.[11] Recently, cyromazine, formulated as 'Trigard'® 75WP, has been shown to be effective in the control of leafminers (Diptera, Agromyzidae) on flowers and vegetables[12–17] as well as the Colorado potato beetle on potatoes.[18]

The present study was initiated to determine the absorption, metabolism, translocation and residues of cyromazine in tomatoes.

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2 MATERIALS AND METHODS

2.1 Test chemicals and reference standards

[*Ring*-U ^{14}C] cyromazine with a specific activity of 67·0 mCi mg^{-1} and radiochemical purity of 97·5%, technical cyromazine with a purity >96%, and inert ingredients of the commercial cyromazine 750 g kg^{-1} WP, 'Trigard'® 75WP, were provided by CIBA, Greensboro, NC, USA. Melamine (2,4,6-triamino-1,3,5-triazine) with a purity >99% was obtained from Aldrich Chemical Company Inc., Milwaukee, WI, USA. Additional standards provided by CIBA were hydroxy-cyromazine (2-hydroxy-4-cyclopropylamino-6-amino-1,3,5-triazine), ammelide (2,4-dihydroxy-6-amino-1,3,5-triazine) and ammeline (2,4,6-trihydroxy-1,3,5-triazine).

2.2 Preparation of plant materials

Seeds of tomato (*Lycopersicon esculentum* Mill., cv. Marion) from Wyatt-Quarles Seed Co., Raleigh, NC,

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TABLE 1
R_f Values of Cyromazine and Metabolites

Compound	R_f Values with TLC solvent systems[a]				
	I	*II*	*III*	*IV*	*V*
Cyromazine	0·58	0·77	0·63	0·56	0·49
Hydroxy-cyromazine	0·0	0·0	0·20	0·0	0·0
Melamine	0·0	0·65	0·0	0·0	0·28
Ammeline	0·0	0·0	0·0	0·0	0·0
Ammelide	0·0	0·0	0·0	0·0	0·0
Unknown #1	0·57	0·69	0·27	0·46	0·29
Unknown #2	0·85	0·88	0·48	0·57	0·61

[a] TLC solvent systems:
I. acetone + methanol + toluene (13 + 6 + 1, by volume).
II. acetone + water (17 + 3, by volume).
III. chloroform + methanol + formic acid + water (75 + 20 + 4 + 2, by volume).
IV. *p*-dioxane + toluene + methanol + ammonium hydroxide (40 + 40 + 30 + 10, by volume).
V. acetonitrile + water (85 + 15, by volume).

USA, were germinated in the greenhouse in plastic trays containing growing media ('Terra-lite'®—a mixture of Canadian Sphagnum peatmoss, horticulture vermiculite and perlite). Individual seedlings were transferred into 10-cm diameter clay pots when they were in the true third-leaf stage (21 days after sowing). Other sets of plants were grown as described in three-week intervals for consecutive experiments.

2.3 Determination of dislodgable residue on tomato leaf surface

Cyromazine 750 g kg^{-1} WP ('Trigard' 75WP) was blended with [^{14}C]cyromazine and the equivalent of 0·75 μg AI (~100 000 dpm) was applied to the terminal leaflet of tomatoes that were in the fifth-leaf stage (three weeks after transplant). Plants were sampled for ^{14}C analysis at 0, 1, 3, 7, 14, 21 and 28 days after treatment. Treated leaflets were washed three times with 10 ml methanol to remove the unabsorbed chemicals from the leaf surface.

In order to determine if any photochemical reactions were responsible for the alteration of cyromazine on the leaf surface, 2·7 μg [^{14}C]cyromazine was applied to glass slides and stored under the same conditions as the tomato plants. The treated slides were each washed with methanol (10 ml) at various time periods after treatment and the radioactivity quantified by using a Packard Model 3330 Liquid Scintillation Spectrometer (LSC).

2.4 Determination of tissue residues

After the methanol wash, treated leaflets and other parts of the plant, including new growth, were homogenized in distilled water in a volume of 10 ml gm^{-1} of plant tissue, and aliquots were taken for combustion in the Harvey (OX-600) Biological Sample Oxidizer (R. J. Harvey Instrument Corp., Hilldale, NJ, USA) for the determination of radioactivity. The homogenate was centrifuged at 7000*g* for 10 min and the radioactivity in the aqueous phase was determined by LSC. The remaining aqueous phase was freeze-dried in a Thermo Vac Freezer-Dryer for subsequent metabolic studies.

2.5 Determination of translocation from treated foliage

[^{14}C]Cyromazine was applied to tomato leaflets as described in Section 2.3 and plants were sampled at 1,3, 7 and 10 days after treatment and exposed to Kodak® X-Ray film SB-5, 8 × 10 in for 10 days before development.

2.6 Isolation of cyromazine and metabolites for instrumental analysis

[^{14}C]Cyromazine 750 g kg^{-1} WP (40·7 mg in 10 ml water; ~7 600 000 dpm) was applied to the terminal leaflets of 18 tomato plants that were in the fifth-leaf stage (three weeks after transplant). Twenty-one days after treatment, the treated leaves were washed with methanol (3 × 10 ml) to remove unabsorbed radioactivity from the leaf surface. The surface washes were combined and concentrated under nitrogen. The methanol-washed leaves were then homogenized in methanol (1 gm in 10 ml methanol) and centrifuged at 7000*g* for 10 min. The supernatant from the entire 18 plant treatments was combined and concentrated. Both

TABLE 2
Persistence of Cyromazine and Metabolites on and in Tomato Foliage[a]

	μg cyromazine equivalent (±SD)		
Days after treatment	*Surface*	*Internal*	*Residual*
0	0·64 (±0·02)	0·10 (±0·01)	0·002 (±0·0005)
1	0·60 (±0·03)	0·13 (±0·03)	0·01 (±0·001)
3	0·55 (±0·05)	0·17 (±0·02)	0·01 (±0·001)
7	0·33 (±0·12)	0·24 (±0·05)	0·02 (±0·004)
14	0·38 (±0·04)	0·29 (±0·02)	0·02 (±0·001)
21	0·40 (±0·02)	0·25 (±0·03)	0·02 (±0·001)
28	0·27 (±0·06)	0·34 (±0·03)	0·02 (±0·001)

[a] 0·75 μg of cyromazine applied as 'Trigard'® 75W formulation.

the surface wash and the homogenate were spotted onto 0·25 mm silica gel 60 F 254 precoated TLC plates (E. Merck, Darmstadt, Germany) for separation. The various radioactive peaks were located using a Berthold LB 2832 Automatic TLC-Linear Analyzer, the silica gel scraped from the plates and the radioactivity eluted with methanol. The methanolic solution was filtered through a BioRad 0·45 μm Prep-Disc Membrane filter and concentrated under nitrogen for instrumental analysis.

TABLE 3
Persistance of ^{14}C-radioactivity on Glass Surfaces[a]

Days after treatment	*Dose recovered (%) (±SD)*	*Lost (%)*
0	99·93 (±0·07)	0·07
1	100·00 (±0·00)	0·00
3	97·18 (±2·83)	2·82
14	91·60 (±0·60)	8·40
21	87·18 (±0·78)	12·82
28	76·60 (±0·64)	23·40

[a] 2·7 μg of cyromazine applied to glass surface.

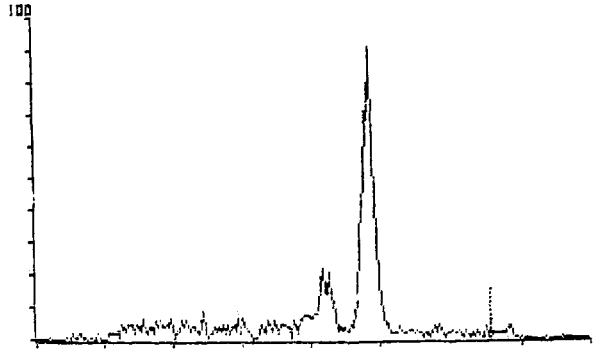

Fig. 1. Radiochromatogram of cyromazine and melamine on glass plates stored in the dark.

2.7 Chromatography and characterization of metabolites

Methanolic solutions of the surface wash were evaporated to dryness under nitrogen, while the aqueous extracts of the plant tissue were freeze-dried. Samples were redissolved in methanol, spotted on TLC plates and co-chromatographed with authentic standards in various solvent systems (Table 1). Known standards were visualized under UV light at 254 nm while the radioactive metabolites were located and quantitated by the TLC linear analyzer.

3 RESULTS AND DISCUSSION

3.1 Fate of cyromazine on tomatoes

Fourteen days after treatment, more than 50% of the applied cyromazine was still present on the surface of

TABLE 4
Cyromazine and Metabolites Found on Glass Plates in the Presences and Absence of Light

	Residue (%)							
Days after application	*Melamine*		*Unknown #1*		*Cyromazine*		*Unknown #2*	
	Light	*Dark*	*Light*	*Dark*	*Light*	*Dark*	*Light*	*Dark*
0	1·7	2·3	2·1	0	89·1	95·7	3·2	0
1	2·0	9·2	2·9	0	88·4	85·0	6·8	0
3	5·6	7·0	4·7	0	81·6	84·7	4·5	0
7	12·7	7·8	15·8	0	60·6	82·7	10·9	0
14	13·0	5·9	15·2	0	53·5	82·9	9·1	0
21	15·9	7·9	23·5	0	48·3	80·0	9·2	0
28	—	8·4	—	0	—	79·6	—	0

TABLE 5
Nature of Cyromazine and its Metabolites on and in Tomato Leaves at Various Times after Treatment

Days after treatment	*Content (%)*[a]							
	Melamine		*Unknown #1*		*Cyromazine*		*Unknown #2*	
	Surface	*Internal*	*Surface*	*Internal*	*Surface*	*Internal*	*Surface*	*Internal*
0	6·40 (±1·11)	1·96 (±1·41)	1·16 (±1·64)	7·70 (±0·64)	85·49 (±2·94)	93·32 (±4·73)	4·08 (±0·85)	0
1	7·14 (±1·23)	1·54 (±1·10)	4·70 (±0·54)	7·08 (±0·67)	82·47 (±3·11)	91·38 (±1·46)	3·34 (±0·66)	0
3	7·89 (±0·37)	1·31 (±0·95)	4·96 (±1·11)	9·38 (±0·94)	78·83 (±2·49)	88·98 (±1·87)	4·62 (±0·51)	0
7	12·87 (±0·64)	2·28 (±0·31)	11·05 (±4·53)	9·90 (±0·94)	63·46 (±9·57)	87·82 (±1·24)	5·38 (±1·13)	0
14	13·60 (±1·96)	2·12 (±0·01)	11·02 (±0·85)	11·20 (±3·71)	65·44 (±2·42)	86·68 (±3·71)	5·68 (±0·62)	0
21	16·34 (±3·05)	1·91 (±0·18)	9·89 (±1·41)	11·14 (±2·46)	65·37 (±4·23)	86·95 (±2·62)	4·53 (±0·27)	0
28	24·09 (±1·75)	1·78	15·97 (±1·55)	11·70 (±1·93)	44·12 (±3·47)	87·42 (±2·82)	6·15 (±1·75)	0

[a] Average of three replicates (±SD).

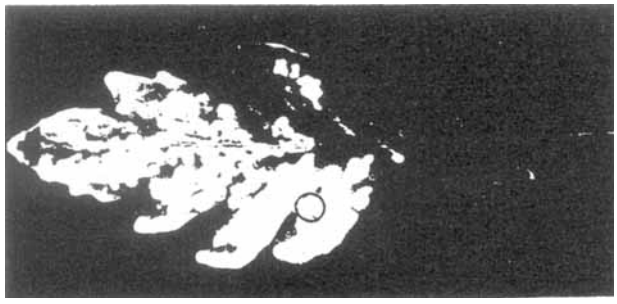

Fig. 2. Autoradiograph of a tomato leaflet to which [^{14}C]cyromazine had been applied seven days earlier.

the tomato leaves. Residues inside the leaf gradually increased with time, and approximately 45% of the applied dose was detected internally 28 days after treatment (Table 2). Seven days after treatment, some radioactivity was detected in parts of the tomato plant that had not been treated. This finding would indicate that cyromazine was absorbed and translocated in the tomato plant, especially since the volatility of cyromazine is low.

3.2 Persistence on glass surface

Cyromazine was quite persistent when applied to glass plates and stored under the same conditions as the treated plants. More than 90% of the applied dose was detected on the surface of the glass 14 days after treatment (Table 3). Only approximately 14% was lost through volatilization, etc. after three weeks.

3.3 Separation and isolation of metabolites

Only two radioactive compounds, melamine and cyromazine, were found in the methanol wash upon removal from glass plates kept in the dark (Fig. 1) whereas four radio-labeled compounds were found on glass plates exposed to light (Table 4). The same four radioactive compounds were found on the surface of the tomato leaves, but only three of them were isolated from within the leaves (Table 5). Unknown #1 was also found both internally and externally while unknown #2 was found only on the surface of the leaves. The amounts of each compound in certain fractions and after periods of time after application are presented in Tables 4 and 5.

Since unknown #2 was found after exposure to light on both glass plates and leaf surfaces, one would expect that the formation of this metabolite was non-enzymatically catalysed by a light reaction. One mass spectrum from a leaf wash had a mass peak at approximately 320 m/z and could represent two cyromazine molecules bridged by an amine group. This molecule would be formed by a free radical mechanism. The partition coefficient of the molecule could be such that penetration was not possible. Additionally, the lack of an increasing or decreasing trend in the amount of unknown #2 on the leaf surface may indicate volatilization of the molecule or a reaction that had run to completion producing a highly stable entity. Unknown #1, on the other hand, was also probably formed by a light reaction of the leaf surface but then subsequently penetrated into the leaf.

The four peaks isolated by TLC were analysed by direct insertion into a Hewlett-Packard 5985 B quadrupole mass spectrometer. The initial peak from TLC was identified as melamine with $m/z = 126$ and the third peak as cyromazine with $m/z = 166$. They were also characterized by co-chromatography with known standards. An attempt to identify the other two unknowns was unsuccessful.

3.4 Movement of cyromazine in tomato tissue

Autoradiographs showed that cyromazine was absorbed and translocated from a treated site to other parts of the tomato plant, both to new growth and parts below the treated area (Fig. 2). This confirmed the movement of radioactivity into parts of the tomato plant that had not been treated, and was detected by sample combustion, as described above.

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